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## Development of Suspension Adaptation, Scale-up cGMP Banking and Cell Characterization Technologies for hESC Lines

### Grant Award Details

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Development of Suspension Adaptation, Scale-up cGMP Banking and Cell Characterization Technologies for hESC Lines

**Grant Type:** Tools and Technologies I

**Grant Number:** RT1-01057

**Investigator:**

<b>Name:</b>	Larry Couture
<b>Institution:</b>	City of Hope, Beckman Research Institute
<b>Type:</b>	PI

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**Human Stem Cell Use:** Embryonic Stem Cell

**Award Value:** \$882,929

**Status:** Closed

### Progress Reports

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**Reporting Period:** Year 1

**View Report**

**Reporting Period:** Year 2

**View Report**

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### Grant Application Details

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**Application Title:** Development of Suspension Adaptation, Scale-up cGMP Banking and Cell Characterization Technologies for hESC Lines

**Public Abstract:**

hESCs represent an important source of cell therapies in regenerative medicine and the study of early human development. A number of hESC-based therapies are nearing clinical trials. To bring these to clinical trials requires the scale-up production, or "banking", of large numbers of the desired hESC cell. The current lack of large scale hESC culture methods presents a serious challenge to ensuring progression of new therapies into clinical testing. In addition, current characterization methods are inadequate to monitor genetic and epigenetic changes that may occur during the long term culture required for banking. Finally, the lack of well characterized hESC research banks limits the comparability of research between laboratories. We propose to address these issues by adapting three representative cell lines to scalable suspensions culture, develop epigenetic and genetic "fingerprinting" methods and generate well characterized Master Cell Banks of the three hESC lines for use by all CIRM investigators.

Procedures typically used in adherent hESC cell banking involve feeder cell layers, undefined media and/or mechanical manipulation. Banking of cell lines for anticipated clinical studies with existing technology is impractical, expensive and time consuming. The development of robust large scale banking technology will accelerate the speed of development of hESC therapeutics. In addition, monitoring pluripotency of hESC cell lines during culture adaptation and banking processes is critical. While the ultimate measure of hESC pluripotency is their ability to form teratomas in animal models, this method is insensitive, time consuming and costly, and is not feasible as an in-process or final product test method. Improved in-process characterization methods with demonstrated correlation to pluripotency are needed. While phenotypic and gene expression parameters have been defined for a number of lines, little is known about the fundamental genetic and epigenetic characteristics of these cells as they are maintained in culture. It is becoming apparent that the self-renewal and differentiation potential of hESCs may be impacted by the genetic and epigenetic status of the cells. Further, it is likely that the genetic and epigenetic status of the cells will be important predictors of safety for clinic studies. Correlating a genetic and epigenetic "fingerprint" of hESC lines during long term cell culture with pluripotency as measured by teratoma formation will provide a novel method of predictive in-process monitoring of cultures during banking and would facilitate comparison of processes between various laboratories.

The unprecedented cGMP cell banking facilities along with the collective expertise of the assembled team, offers an opportunity to advance the hESC field by establishing suspension adaptation techniques, epigenetic fingerprinting, cGMP scale-up processes & cGLP banking of hESC lines not fundable under current NIH rules and policies.

**Statement of Benefit to California:**

An advantage of human embryonic stem cells (hESCs) in research is their ability to self-renew indefinitely. hESCs can provide an inexhaustible source of well-defined human cells and represent an important source of material for therapies in regenerative medicine, for the study of early human development and for many other areas of biomedical research. For the first time, it will be possible to grow large quantities of hESC that can meet the requirements of the FDA. As a number of hESC-based therapies developed in California near clinical trials, there is a pressing need for a source of high quality, well defined hESCs. However, translating bench science into clinical reality involves large scale cell banking and predictive cell characterization and release testing that correlate well with the pluripotent properties of these cells. Current procedures for the culture of these cells are limited to work from research laboratories where the focus is on research and not on methods for large scale production or the Good Manufacturing Practices (cGMP) required to meet FDA standards. While several groups have produced small scale cell banks using current technologies, the methods are neither practical or cost effective, and are not amendable to large the scale expansion and banking required for clinical development.

One of the major limitations to growing large amounts of hESCs is that current procedures require manual isolation of cell colonies –a time consuming process. A second limitation is that hESC grow only when adhered to surfaces with “feeder” cells and/or specialized coatings. As different laboratories employ their own methods, each may bias cells to be slightly different from the parent cell line. Lack of standardization of cell lines used by researchers is a concern in comparing research across the field. We propose to address these issues by 1. developing procedures to adapt three hESC lines to suspension culture, 2. create well characterized cell banks of these lines, using GMP processes suitable for preclinical and clinical use and 3. develop epigenetic and genetic fingerprinting for cell bank testing and monitoring of cell lines.

This proposal brings together nationally recognized leaders in cell culture, assay development and cGMP banking to develop hESC culture conditions that can be scaled up, to design and implement novel assays for characterizing cells obtained at each stage of production, and to create Master Cell Banks of important hESC lines. These cell banks and assay methodologies will be available to all CIRM investigators and will be a key resource for all investigators in the state of California. The unprecedented cGMP cell banking facility along with the collective expertise of the assembled team, offers an opportunity to advance the hESC field by establishing suspension adaptation techniques, epigenetic fingerprinting, cGMP processes & cGLP banking of hESC lines not funded nor fundable under current NIH policies.

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